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ISOLATION OF BIOPIGMENTS FROM FOUR BACTERIAL STRAINS AND THEIR APPLICATION IN TEXTILE DYEING

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Abstract

The objective of this study was the isolation and characterization of pigmented bacteria, with a focus on exploring their pigment-producing capacities and assessing the practical applicability of these pigments in textile dyeing procedures. Four distinct pigmented bacterial strains—*Chryseobacterium meningosepticum* (Pf5), *Proteus vulgaris* (HC), *Aeromonas hydrophila* (Pf4), *Halomonas* sp. (AST)—were carefully isolated and subjected to an investigation into optimized culture and media variables. The identified optimal conditions for both growth and pigment production included the use of peptone broth media at 37°C, pH=7, 2-4% salination in the growth media, under shaking and dark conditions. The biopigments were subsequently extracted in organic solvents, including diethyl ether, chloroform, ethanol, and methanol. UV/Vis spectrophotometry revealed peak absorbance at 440-450nm for pigments extracted using diethyl ether and chloroform. The antibacterial activity assessment confirmed the presence of antibacterial substances within these biopigments. Furthermore, the extracted pigments underwent comprehensive testing for their coloring potential on diverse materials such as fabrics, soap, cotton, and tissue paper, demonstrating a non-degradable colorant. These findings highlight the potential applications of these bacteria in advancing sustainable and environmentally friendly dyeing processes within the textile industry.

Keywords: Biopigments, Textile Dyeing, Culture Conditions, Organic Solvents, Chromogenic, Bacteria, Green Chemistry.

1. INTRODUCTION

The extensive production and utilization of synthetic dyes across diverse industries present substantial environmental challenges, intensifying concerns related to air pollution, soil contamination, and the degradation of water resources[1],[2]. The manufacturing process of synthetic dyes is a noteworthy contributor to air pollution, predominantly due to carbon emissions[3]. Also, the harmful by-products arising from dye synthesis exert adverse effects on soil and water resources, leading to environmental

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deterioration[4],[5]. In soil, the accumulation of toxic residues originating from synthetic dyes poses risks to both agricultural productivity and overall terrestrial ecosystem health. The manifestation of microorganism toxicity further underscores the profound consequences associated with the usage of synthetic dyes[6],[7],[8]. In response to growing ecological, there is an imperative to explore alternative methods for dye production that prioritize safety, mitigate environmental effects linked to conventional synthetic dye manufacturing, and the absence of adverse effects on human health[9].

An eco-friendly approach and sustainable model involves the substitution of synthetic dyes with bio-colorants, employing biotechnology methods for synthesizing bacterial pigments[10],[11]. This approach not only addresses environmental concerns but also offer various advantages, including consistent availability, cost-effectiveness, sustainability, and high production efficiency[12],[13]. Biotechnologically obtained dyes, or biopigments, constitute biodegradable compounds sourced from a diverse array of organisms, including fungi [14],[15], virus [16] and bacteria [17],[18],[19],[20]. Significantly, bacterial pigments present unique benefits compared to their plant-derived counterparts, such as rapid growth, minimal cost for culture mediums, process adaptability during fermentation, and resilience to diverse weather conditions[21],[22]. Numerous studies have validated the efficacy of naturally produced microbial pigments as potent colorants, emphasizing their substantial potential to make significant contributions to environmental sustainability in the domain of dye production[16],[23],[24].

Based on the growing demand for environmentally sustainable alternatives[25],[26],[27], this study systematically investigates the isolation, screening, and characterization of pigment-producing bacteria across varied ecological habitats, encompassing soil, industrial discharges, damping sites, compost, spoiled fruits and vegetables, restaurants' discard water, and hospitals. The study also focuses on the isolation and cultivation of specifically chosen bacterial strains, with an emphasis on optimizing biopigment production. The extracted biopigments undergo the characterization process, involving a thorough analysis of their chemical composition, stability, and spectral properties. To assess their practical viability, the characterized biopigments are applied as colorant agents across diverse organic fabrics, providing valuable insights into their dyeing potential and stability under various conditions. This multidimensional approach integrates microbiological, biochemical, and textile engineering perspectives, thereby furnishing substantial insights into the evolving landscape of naturally-derived pigment utilization.

2. MATERIALS AND METHODS

2.1 Chemicals and Chemistry of Culture Media

Reagent-grade chemicals, such as ethanol (EtOH), chloroform (Chl), diethyl ether (Et2O), and methanol (MeOH), were acquired from Lab Chem Scientific in Lahore, Pakistan. Silica gel plates (60 for thin-layer chromatography, TLC) were also sourced from the same supplier. The National Institute of Health (NIH) in Islamabad, Pakistan, provided the agar

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plates and culture medium. The L-broth medium composition comprised of Tryptone (10g), yeast extract (5g), NaCl (5g), and Agar (20g/1L distilled water, pH-7).

2.2 Collecting samples: Techniques and procedures

Soil specimens were acquired from distinct locations, namely Punjab University Bridge 4 (31°30'N, 74°16'E) and Botanical Garden (31°30'N, 74°18'E), agricultural fields of Kasur (31°10'N, 74°28'E) and River Ravi Lahore (31°34'N, 74°48'E), following sterile protocols. The topmost soil layer (up to 15 cm) was excluded to remove any potential surface contaminants. Subsurface soil samples (20-50 grams each) were carefully gathered from each core, placed securely in sterile containers, sealed, and subsequently transferred to zip-lock bags for transportation to the laboratory. Upon reaching the laboratory, these samples were attentively stored at 20 °C, awaiting further processing.

2.3 Soil bacteria isolation

In the preliminary phase, subconfluent bacterial colonies were cultured to facilitate the growth of singular species populations. Following this, agar plates were employed to ensure uniform distribution and growth of individual colonies. The selection of bacterial colonies displaying distinct morphological features was performed, and purification was achieved through streak plate techniques. The conclusive identification of 140 unique bacterial species was established utilizing a combination of biochemical and molecular identification methods.

2.4 Identification of bacterial strains through biochemical assays

Bacterial isolates underwent biochemical characterization using the Quick Test Strip 12 (QTS-12) bacterial identification kit sourced from DESTO Laboratories, Karachi, Pakistan[28]. This kit applied standard biochemical methods, assessing 14 distinct reactions. Micro-tubes containing dehydrated enzymatic substrate from QTS-12 were inoculated with pure saline bacterial cultures, and the results were documented following an 18-24 hour incubation period.

2.5 Identification and screening of pigmented bacteria

From the pool of 140 isolates subjected to careful examination, a subset of 25 isolates displayed the capability for pigment production. Specifically, these 25 isolates underwent growth in liquid media, and their potential for pigment production was assessed through both visual observation and spectrophotometric analysis. Subsequently, an in-depth examination was conducted on the color stability of these isolates, revealing that 21 maintained minimal alterations in color stability even after 48 hours of growth in liquid media. Consequently, only four pigmented bacteria—namely, Chryseobacterium meningosepticum (Pf5), Proteus vulgaris (HC), Aeromonas hydrophila (Pf4) and Halomonas sp. (AST)—were chosen for further comprehensive analysis.

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2.6 Morphological profiling of pigmented bacteria

The four pigmented bacterial isolates were subjected to streaking on L-agar plates, yielding individual colonies for each isolate and enabling a comprehensive examination of their characteristics, including shape, color, elevation, size, and transparency. In addition, Gram staining was applied to facilitate the investigation of cellular morphology for the bacterial isolates, providing valuable insights into their structural composition. Furthermore, a motility test was executed using the wet mount slide depression method, delivering crucial information regarding the isolates' capability to exhibit movement. This integrated approach ensured an intricate analysis of both macroscopic and microscopic features, significantly contributing to basic understanding of the pigmented bacterial isolates.

2.7 Physiological profiling of pigmented bacteria

All bacterial isolates underwent incubation in L-broth medium at 37°C, where the growth was assessed at intervals of 24, 48, 72, 96, and 120 hours post-incubation. Furthermore, to explore the impact of environmental variables, the growth of the isolates was studied under the same incubation conditions but with the additional factors of continuous shaking and exposure to light at an intensity of 10,000 lux. To explore the adaptability and responses of the isolates to diverse environmental conditions, the study further investigated the influence of varying pH levels (ranging from pH 3 to 11), temperatures (at 20°C, 37°C, and 45°C), and salinity levels (with NaCl concentrations spanning 2%, 4%, 6%, and 8%) on the optimal growth of bacterial isolates. Moreover, the impact of these aforementioned variables—light, pH, temperature, and salinity—on pigment production was also explored.

2.8 Optimization of pigment synthesis

A diverse set of growth conditions, including static, shaking, light exposure, and darkness, were carefully selected to explore their impact on bacterial growth and pigment production. Furthermore, the study incorporated a range of physiological conditions, encompassing temperature variations (20°C, 37°C to 45°C), pH variations (at 3, 5, 7, 9, and 11), and NaCl concentrations (at 2%, 4%, 6%, and 8%). The study observed and analyzed bacterial growth dynamics and pigment synthesis at time intervals of 24, 48, 72, 96, and 120 hours post-incubation.

2.9 Extraction and characterization of bacterial pigment

Following the optimization of pigment production from the selected bacterial isolates, organic solvents were employed for pigment extraction. In the initial phase, the bacterial isolates were inoculated in 100 mL peptone broth, allowing them to incubate until the production of biocolors, typically observed after one week. Subsequently, pigmented broth cultures underwent centrifugation at 9000 rpm for 20 minutes, and the resulting supernatants were combined with organic solvents in a 1:1 ratio for efficient pigment extraction. The mixtures were left undisturbed until complete solvent evaporation.

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The extracted pigments underwent a thorough calorimetric examination using a Shimadzu UV-1601PC UV-Visible, Scanning Spectrophotometer, operating within the wavelength range of 350-750 nm to establish absorption spectra. Additionally, qualitative analysis was conducted through thin-layer chromatography, utilizing a mixture of petroleum ether and acetone (v/v 2:1) as mobile solvents.

In evaluating antibacterial activities, the four bacterial isolates were subjected to the disc diffusion methodology against five distinct bacterial strains from different genera, namely Bacillus pumilus, Clostridium butyricum, Pseudomonas sp., Bacillus safenesis, and Bacillus cereus. The resulting inhibition zones were accurately evaluated, and the precise diameter of each zone, expressed in millimeters, was recorded. This approach aimed to provide a thorough assessment of the extracted pigments and their potential antibacterial properties.

2.10 Application of bacterial pigments as dyeing agents

The stability and dyeing capabilities of the extracted pigments were rigorously evaluated. The pigments, dissolved in water, were systematically applied to small fabric specimens measuring 5 x 5 cm2, representing diverse materials such as cotton, nylon, silk, lawn, tissue paper, and soap[29]. To assess dyeing potential and stability, these specimens underwent exposure to elevated temperatures through a mixture of distilled water (20 ml containing 3% agar) and varying pigment concentrations (ranging from 100 to 500 µg/ml) at boiling temperature[30]. Furthermore, the dyed specimens were subjected to cycles of washing to assess the color retention properties under repeated laundering conditions. Simultaneously, rubbing tests were conducted to evaluate the resistance of the dyed fabrics against abrasion and wear. This examination aimed to provide in-depth insights into the practical utility and robustness of the extracted pigments in real-world textile applications.

3. RESULTS

3.1 Pigmented bacteria characterization

In this study, four bacteria— Chryseobacterium meningosepticum (Pf5), Proteus vulgaris (HC), Aeromonas hydrophila (Pf4) and Halomonas sp. (AST)—were systematically examined for their pigment-producing capabilities. Morphological and physiological characterization of these pigmented isolates revealed the following characteristics. The colony and cell morphology of the bacterial isolates were carefully observed. Chryseobacterium meningosepticum (Pf5) displayed round colonies with a concave shape, measuring 1 mm, and dentate margins. Also, Pf5 exhibited a distinctive yellow color and was identified as gram-positive. The cells in Pf5 isolates were non-motile and displayed a spiral morphology. Proteus vulgaris (HC) featured oval-shaped colonies with entire margins, a sea-green color, concave elevation, and a size of approximately 8 mm. Aeromonas hydrophila (Pf4) presented rounded colonies with entire margins, a brownish color, convex elevation, and a size of 2 mm. Finally, colonies of Halomonas sp. (AST)

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were rounded with entire margins, displaying a lemon color, convex elevation, and measuring around 5 mm. Microscopic examination of cell morphology revealed that the later three isolates were gram-negative, bacillus-shaped, and arranged in isolated forms.

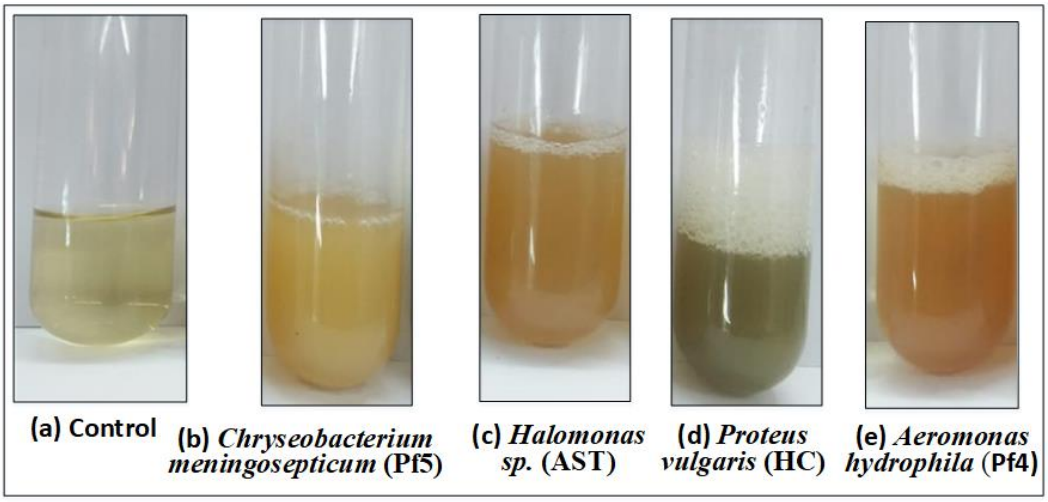

Fig 1: Pigment produced by bacterial isolates. (a) Control, (b) *Chryseobacterium meningosepticum* (Pf5), (c) *Halomonas* sp. (AST), (d) *Proteus vulgaris* (HC), and (e) *Aeromonas hydrophila* (Pf4),

Table: Biochemical characterization of the four bacterial strains

Reaction	HC	Pf5	Pf4	AST
H_2S production	-	-	+	+
Voges-Proskauer	+ + +	+ + +	+ + +	+ + +
Catalase Test	+ + +	+ + +	+ + +	+ + +
Methyl Red Test	-	+ + +	-	-
Indole Test	+ + +	+ + +	-	+ + +
Lactose Fermentation Test	-	-	-	-
KOH Test	+ +	+ +	+	+
Motility Test	Non-motile	Non-motile	Motile	Non-motile
Citrate Utilization Test	+ + +	+ + +	+ +	+ + +
+ : moderate activity, +++: high activity, - : no activity				

3.2 Growth condition optimization

Bacterial isolates were subjected to static incubation, and their growth dynamics were investigated. HC, Pf5, and AST demonstrated exponential growth after 96 hours, while Pf4 reached its peak at 72 hours, followed by a subsequent decline (Figure 2a). This growth pattern remained consistent in shaken broth cultures (Figure 2b). In the presence of light, all isolates (HC, Pf5, Pf4, and AST) exhibited significant growth after 120 hours (Figure 2c). Conversely, in the absence of light, minimal growth was observed for all isolates up to 48 hours, followed by exponential growth after 120 hours (Figure 2d). This detailed analysis of growth patterns provides valuable insights into how these bacterial isolates respond to various incubation conditions.

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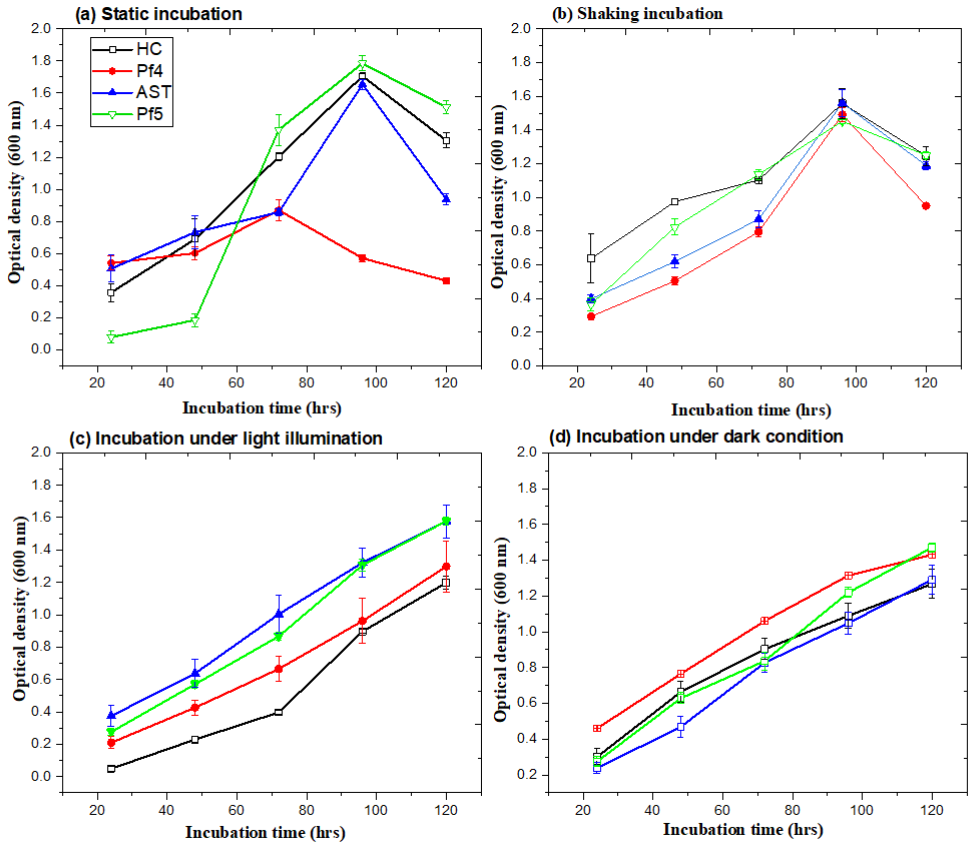

Fig 2: Growth of four bacterial strains at 120 hours with incubation under (a) static, (b) shaking, (c) light, and (d) dark conditions

Bacterial growth was examined at various temperatures, encompassing 20ºC, 25ºC, 37ºC, and 45ºC (Figure 3). At 20ºC, isolates demonstrated their peak growth after 120 hours of incubation, maintaining sustained growth without decline even after five days. In contrast, at 25ºC, HC exhibited its maximum bacterial growth after 96 hours, followed by a subsequent decline, while Pf5, Pf4, and AST achieved their maximal growth after 120 hours, sustaining without decline up to the same incubation period. Furthermore, at 37ºC, HC, Pf4, and AST reached their peak growth after 96 hours, followed by a gradual decline until 120 hours, whereas Pf5 attained its maximum growth after 120 hours. At 45ºC, AST displayed minimal growth after 96 hours, with a subsequent decline evident after four days. Conversely, HC, Pf5, and Pf4 exhibited negligible growth at this elevated temperature. These findings underscore the significance of 37 ºC as the optimal temperature for bacterial growth.

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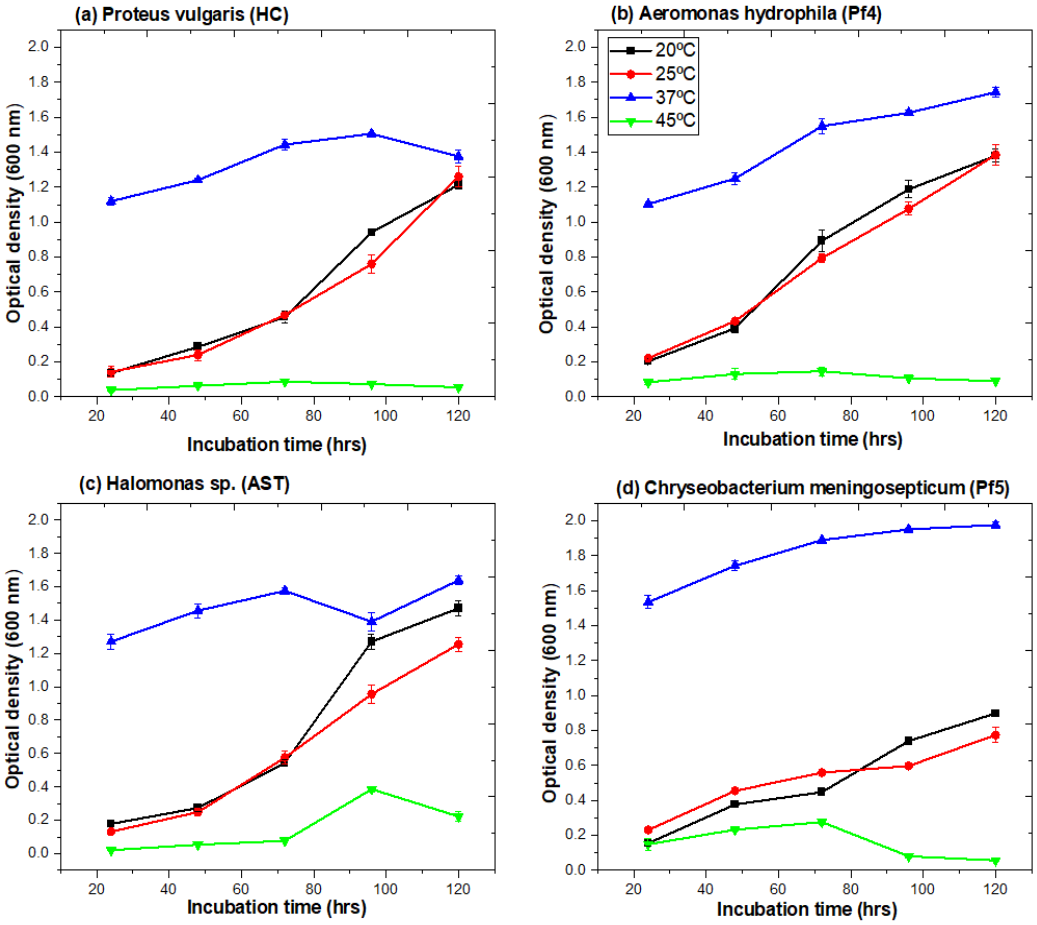

Fig 3: Temperature-dependent growth of (a) Proteus vulgaris (HC), (b) Aeromonas hydrophila (Pf4), (c) Halomonas sp. (AST), and (d) Chryseobacterium meningosepticum (Pf5)

The systematic exploration of varying pH levels (3, 5, 7, 9, and 11) on isolate growth revealed distinctive responses (Figure 4). Under acidic conditions (pH 3 and pH 5), all four bacteria exhibited minimal growth, except for Pf5, which displayed moderate growth at pH 5. At pH 7, all isolates showcased maximal growth after 120 hours of incubation. Furthermore, at pH 9, exponential growth was observed for all isolates after 96 hours, followed by a subsequent decline in growth after 120 hours, indicating the bacterial growth decline phase. Similarly, at pH 11, all isolates reached peak bacterial growth after 96 hours, followed by a decline recorded on the fifth day. Hence, it is apparent that pH 7 and pH 9 are more favorable for bacterial growth, highlighting the isolates' preference for neutral and alkaline environments.

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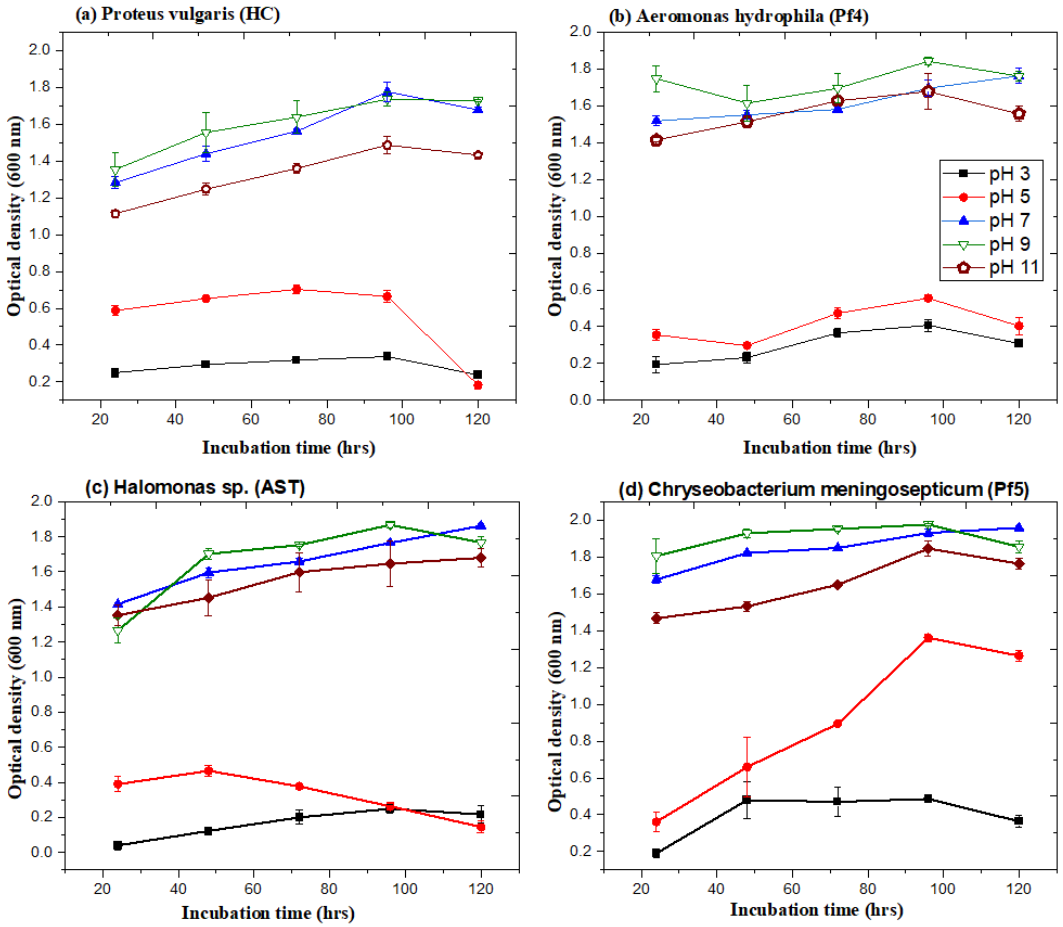

Fig 4: pH-dependent growth of (a) Proteus vulgaris (HC), (b) Aeromonas hydrophila (Pf4), (c) Halomonas sp. (AST), and (d) Chryseobacterium meningosepticum (Pf5)

Different concentrations of NaCl (2%, 4%, 6%, and 8%) were introduced into the growth medium, resulting in diverse impacts on bacterial growth. In the 2% NaCl medium, there was an initial growth increase, followed by a decline after 48 hours for isolate Pf5. Conversely, isolates HC and Pf4 exhibited robust growth after 96 hours, followed by a subsequent decrease. AST displayed peak growth after 72 hours, followed by a decline. In the 4% NaCl medium, all bacterial isolates exhibited significant growth after 96 hours, followed by a decline. Moreover, in the 6% NaCl medium, Pf5 displayed maximal growth after 48 hours, followed by a decline, while Pf4 exhibited peak growth after 72 hours, followed by a decrease. HC and AST showed exponential growth after 96 hours, followed by a decline. Additionally, in the 8% NaCl medium, HC, Pf5, and AST exhibited exponential growth after 48 hours, followed by a subsequent decrease. Pf4 displayed maximum growth after 96 hours, followed by a decline on the fifth day. It is evident that

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lower salt concentrations, specifically 2% and 4%, are more conducive to maximal bacterial growth compared to higher salt concentrations.

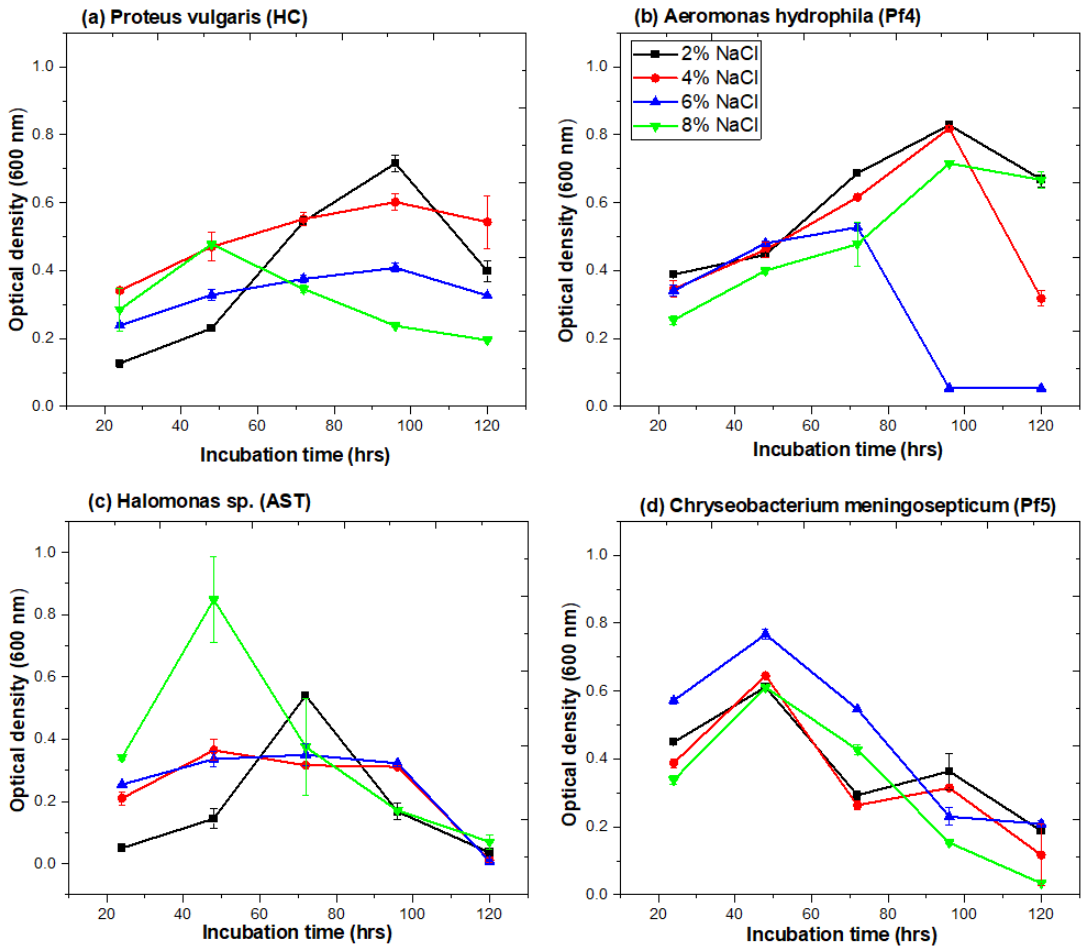

Fig 5: Calcination-dependent growth of (a) Proteus vulgaris (HC), (b) Aeromonas hydrophila (Pf4), (c) Halomonas sp. (AST), and (d) Chryseobacterium meningosepticum (Pf5)

3.3 Bacterial pigment characterization

Isolates were subjected to pigment extraction using diverse organic solvents, and UV-Vis spectrophotometry indicated that the most effective solvents were diethyl ether and chloroform (Figure 6). Specifically, HC displayed maximal pigment content in Chl, moderate in EtOH and MeOH, and minor in Et2O. Similarly, Pf5 and AST exhibited significant pigment content in Chl and Et2O, with moderate content in EtOH and MeOH extracts. Pf4 exhibited consistent results across all organic solvents. HC, Pf5, and AST demonstrated maximum pigment production at 450nm in Et2O, while EtOH and MeOH

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extracts exhibited maximal absorbance for pigment content at 350nm. Overall, pigment production in EtOH and MeOH extracts did not attain the same prominence as pigments extracted in Chl, and Et2O.

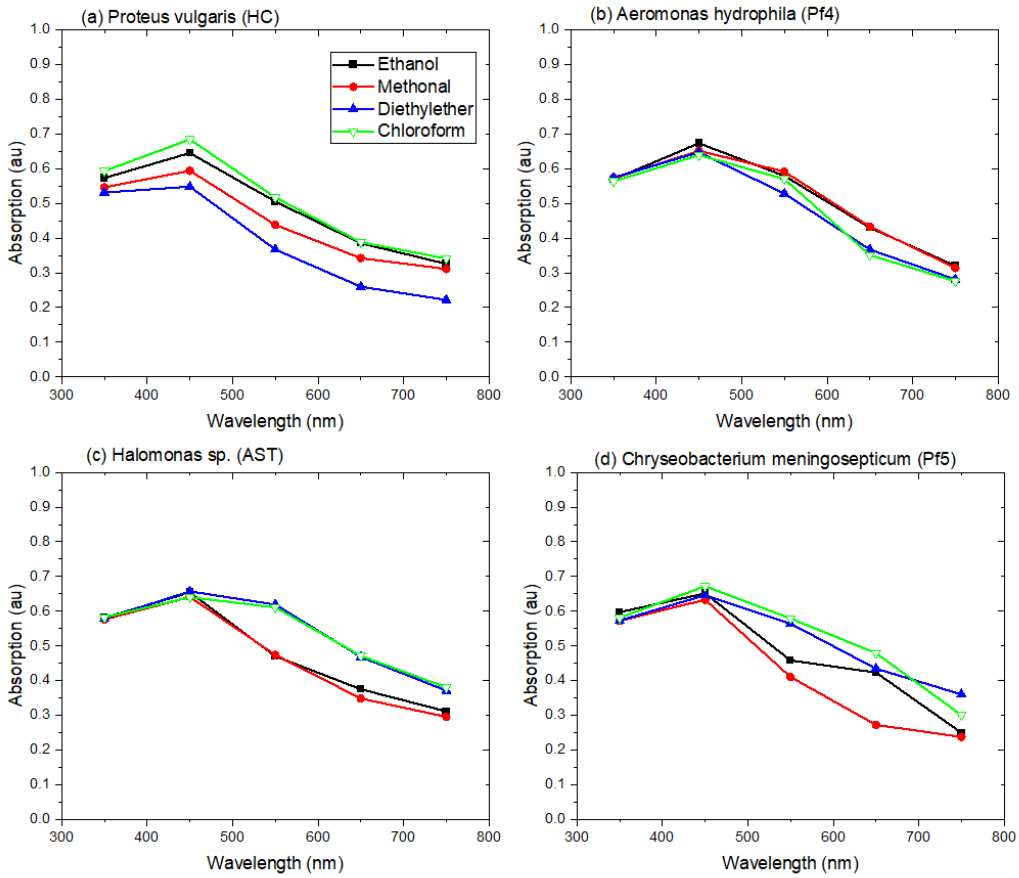

Fig 6: Absorption spectra (a) Proteus vulgaris (HC), (b) Aeromonas hydrophila (Pf4), (c) Halomonas sp. (AST), and (d) Chryseobacterium meningosepticum (Pf5)

Antibacterial activity of isolates was also observed against five pathogenic strains. The isolate HC showed inhibition zone of 0.1, 0.8 and 0.7mm against Pseudomonas sp., Bacillus safensis and Bacillus pumilus respectively. Similarly, Pf4 exhibited antibacterial activity against Pseudomonas sp., Bacillus safensis, Bacillus safensis and Bacillus pumilus with inhibition zone as 0.7, 1.6, 0.8 and 0.7mm respectively. AST formed clear inhibition zone of 1.8, 0.8 and 0.7mm against Clostridium butyricum, Bacillus safensis and Bacillus pumilus respectively.

Thin layer chromatographic (TLC) analysis for extracted pigments has shown the presence of pigmented compounds. The Rf values obtained for isolates HC, Pf4, Pf5 and AST were 0.14, 0.16, 0.15 and 0.17 respectively. Clear pigmented spots were observed for all three isolates.

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3.4 Extracted pigments for dyeing applications

The pigments extracted were employed as dyeing agents and applied to a range of materials, including cotton, tissue paper, and soap, to evaluate their dyeing effectiveness and durability. It was noted that the dyeing potential and stability were superior for pigments extracted in Et2O and Chl, in contrast to extracts in EtOH and MeOH, which were more suitable for dyeing fabrics and cotton only. Biopigments extracted from Pf5 and HC demonstrated optimal dyeing potential for various materials, including cotton, tissue paper, and diverse fabrics (cotton, lawn, silk, and wool), while Pf4 and AST exhibited significant dyeing capability for cotton and tissue paper (Figure 7). Soap exhibited moderate pigmentation with reduced intensity, and original color loss was observed, manifesting in a brownish hue for all isolates rather than retaining their initial pigmented colors. Assessment of dyed cotton, tissue paper, soap, and fabrics under sunlight revealed no pigment degradation at elevated temperatures. The different fabrics used for dyeing purposes retained their color even after washing. Additionally, the degradability of biopigments was assessed using boiling temperature. Maximum coloring potential was identified at 500µg/ml of pigment, with no pigment degradation at boiling temperature, underscoring the stability and viability of the pigments.

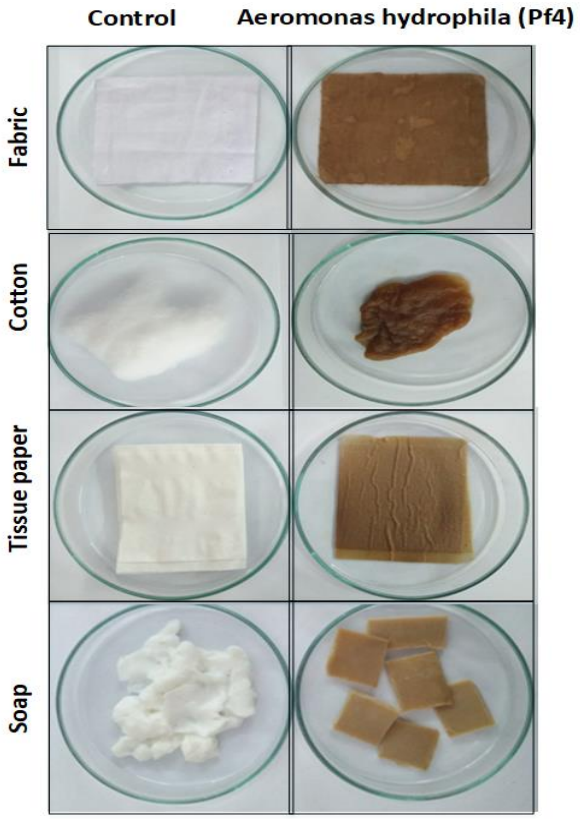

Fig 7: Dyeing potential of Aeromonas hydrophila (Pf4) on fabric, cotton, tissue paper and soap

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4. DISCUSSION

The chromogenicity and related attributes of Chryseobacterium meningosepticum (Pf5), Proteus vulgaris (HC), Aeromonas hydrophila (Pf4), and Halomonas sp. (AST) have not been extensively studied. Hence, the aim of this study was to isolate these bacterial strains, explore their pigment-producing capacities, and assess the practical applicability of these pigments in textile dyeing procedures.

The present study also focused on optimizing culture and media variables to promote growth and pigment production in the four bacterial strains. To achieve this, the optimal experimental conditions included the utilization of peptone broth media, maintaining a temperature of 37°C, a pH level of 7, and incorporating NaCl concentration ranging from 2% to 4% in the growth media. Furthermore, the study observed that bacterial growth and pigmentation were more pronounced in the absence of light compared to the presence of light, and shaking conditions proved more conducive than static conditions[31],[32],[33].

These findings are consistent with the outcomes of previous studies. For example, Chaudhari isolated the bacterium Planococcus maritimus, a potent producer of orange pigment, and found optimal proliferation in a medium containing 0.5-1.0% NaCl, with the pH of the medium adjusted between 6-7, and incubated at 37°C[34]. Similarly, it has been reported that maximum pigment production by bacteria occurred at 37°C after a 72-hour incubation period[30]. Additionally, pH levels between 6-8 and salt concentrations of 4-8% were reported as optimal for both the growth and pigment production by bacteria[35].

Our study of the extraction pigments revealed the highest absorbance at 440-450nm when Et2O and Chl served as extraction solvents, in contrast to EtOH and MeOH extracts. This outcome aligns with the findings of previous studies, where MeOH and acetone were utilized for bacterial pigment extraction, reporting maximum absorbance in the 400-500nm wavelength range[36]. In contrast, maximum absorbance was reported in the 600-700nm range using Chl as the extraction solvent[34]. It was suggested that the isolated pigments might be carotenoids, as indicated by their maximum absorbance spectra (λmax) at 440nm, a characteristic feature of carotenoids [35]. Preliminary results from our study indicated a close resemblance of the extracted biopigments to carotenoids, with maximum absorbance spectra at 440-450 nm[37]. Furthermore, TLC analysis confirmed the presence of pigmented compounds in the extracted biopigments. Additionally, the antibacterial assay underscored the potential of the extracted pigments, hinting at the presence of antibacterial agents capable of inhibiting microbial growth.

Following the characterization of the extracted biopigments, their coloring capabilities were assessed by applying them as dyeing agents on various textile materials[38],[39]. The results yielded promising outcomes in terms of the dyeing potential and color stability of the biopigments. The dyed fabrics underwent exposure to intense sunlight and washing, yet the stability of the pigments affirmed their feasibility as effective dyeing agents[40],[41],[42]. These findings are consistent with the past studies, which reported a yellow pigment extraction from Micrococcus luteus as a dyeing agent for paper, candles,

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soap, and fabrics, achieving promising results. Similarly, another study explored the application of the pigment prodigiosin extracted from Serratia sp. in the textile industry as a natural dye, demonstrating its effectiveness in imparting red color to various textiles[43]. Additionally, a vibrant red pigment from Vibrio sp. was characterized and proposed its use as a dye in various fibers, including wool, nylon, and silk[44]. Similar findings were reported emphasizing the potentiality of pigmented bacteria as dyeing additives [45]. Consequently, the present study advocate for the utilization of biopigments as natural sources of pigmentation in the textile industry.

5. CONCLUSIONS

The bacterial strains, including Chryseobacterium meningosepticum (Pf5), Proteus vulgaris (HC), Aeromonas hydrophila (Pf4), and Halomonas sp. (AST), displayed interesting chromogenic characteristics and demonstrated the synthesis of biopigments. The results of this study highlight the potential of these bacterial strains as an environmentally friendly and sustainable source for biopigment production. Optimal biopigment yields were achieved under conditions of 37°C temperature, pH 7, 2-4% NaCl concentrations, and absence of light. These biopigments present a viable option for utilization in the textile industry as natural colorants, providing an eco-friendly substitute for synthetic dyes and contributing to the reduction of environmental impact. This research adds to the existing body of knowledge dedicated to exploring sustainable solutions across various industries, emphasizing the significance of utilizing natural resources for pigment production.

References

1) Moons, P. , Michiels, C. W. , and Aertsen, A. Bacterial interactions in biofilms bacterial interactions in biofilms. Critical Reviews in Microbiology 2009, 35, 157–168.

2) Mee, M. T. , Collins, J. J. , Church, G. M. , and Wang, H. H. Syntrophic exchange in synthetic microbial communities. Proceedings of the National Academy of Sciences 2014, 111, E2149–E2156.

3) Rousk, J. , Bååth, E. , Brookes, P. C. , Lauber, C. L. , Lozupone, C. , Caporaso, J. G. , Knight, R. , and Fierer, N. Soil bacterial and fungal communities across a pH gradient in an arable soil. ISME Journal 2010, 4, 1340–1351.

4) Bo, A. B. , Kim, J. D. , Kim, Y. S. , Sin, H. T. , Kim, H. J. , Khaitov, B. , Ko, Y. K. , Park, K. W. , and Choi, J. S. Isolation, identification and characterization of Streptomyces metabolites as a potential bioherbicide. PLoS ONE 2019, 14, e0222933.

5) Asker, D. High throughput screening and profiling of high-value carotenoids from a wide diversity of bacteria in surface seawater. Food Chemistry 2018, 261, 103–111.

6) Venil, C. K. , Zakaria, Z. A. , and Ahmad, W. A. Bacterial pigments and their applications. Process Biochemistry 2013, 48, 1065–1079.

7) Malik, K. , Tokkas, J. , and Goyal, S. Microbial Pigments : A review. International Journal of Microbial Resource Technology 2012, 1, 361–365.

8) Joshi, V. , Attri, D. , and Bhushan, S. Microbial pigments. Indian Journal of Biotechnology 2014, 2, 362–369.

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DOI: 10.5281/zenodo.13358020

9) Hibbing, M. E. , Fuqua, C. , Parsek, M. R. , and Peterson, S. B. Bacterial competition: Surviving and thriving in the microbial jungle. Nature Reviews Microbiology 2010, 8, 15–25.

10) Ghoul, M. and Mitri, S. The Ecology and Evolution of Microbial Competition. Trends in Microbiology 2016, 24, 833–845.

11) Janowski, D. and Leski, T. Landscape-scale mapping of soil fungal distribution: proposing a new NGS-based approach. Scientific Reports 2023, 13, 10280.

12) Li, S. peng , Wang, P. , Chen, Y. , Wilson, M. C. , Yang, X. , Ma, C. , Lu, J. , Chen, X. yong , Wu, J. , Shu, W. sheng , and Jiang, L. Island biogeography of soil bacteria and fungi: similar patterns, but different mechanisms. ISME Journal 2020, 14, 1886–1896.

13) Yu, K. , Hoogen, J. Van Den , Wang, Z. , Averill, C. , Routh, D. , Smith, G. R. , Drenovsky, R. E. , Scow, K. M. , Mo, F. , Waldrop, M. P. , Yang, Y. , Tang, W. , Vries, F. T. De , Bardgett, R. D. , Manning, P. , Bastida, F. , Baer, S. G. , Bach, E. M. , Garcia, C. , Wang, Q. , Ma, L. , Chen, B. , He, X. , Teurlincx, S. , Heijboer, A. , Bradley, J. A. , and Crowther, T. W. The biogeography of relative abundance of soil fungi versus bacteria in surface topsoil. Earth System Science Data 2022, 14, 4339–4350.

14) Agboyibor, C.; Kong, W. B.; Chen, D.; Zhang, A. M.; and Niu, S. Q. Monascus pigments production, composition, bioactivity and its application: A review. 2018.

15) Cho, Y. J. , Park, J. P. , Hwang, H. J. , Kim, S. W. , Choi, J. W. , and Yun, J. W. Production of red pigment by submerged culture of Paecilomyces sinclairii. Letters in Applied Microbiology 2002, 35, 195–202.

16) Dawson, T. L. Biosynthesis and synthesis of natural colours. Coloration Technology 2009, 125, 61–73.

17) Mohammed, S. J. and Luti, K. J. K. In A kinetic model for prodigiosin production by Serratia marcescens as a bio-colorant in bioreactor, 2020.

18) Galal, G. F. and Ahmed, R. F. Using of Some Agro-industrial Wastes for Improving Carotenoids Production from Yeast Rhodotorula glutinis 32 and Bacteria Erwinia uredovora DSMZ 30080. Microbiology Research Journal International 2020, 30, 15–25.

19) Majumdar, S. , Priyadarshinee, R. , Kumar, A. , Mandal, T. , and Dasgupta Mandal, D. Exploring Planococcus sp. TRC1, a bacterial isolate, for carotenoid pigment production and detoxification of paper mill effluent in immobilized fluidized bed reactor. Journal of Cleaner Production 2019, 211, 1389–1402.

20) Patthawaro, S. , Lomthaisong, K. , and Saejung, C. Bioconversion of Agro-Industrial Waste to Value-Added Product Lycopene by Photosynthetic Bacterium Rhodopseudomonas faecalis and Its Carotenoid Composition. Waste and Biomass Valorization 2020, 11, 2375–2386.

21) Órdenes-Aenishanslins, N. , Anziani-Ostuni, G. , Vargas-Reyes, M. , Alarcón, J. , Tello, A. , and Pérez-Donoso, J. M. Pigments from UV-resistant Antarctic bacteria as photosensitizers in Dye Sensitized Solar Cells. Journal of Photochemistry and Photobiology B: Biology 2016, 162, 707–714.

22) Sajjad, W. , Din, G. , Rafiq, M. , Iqbal, A. , Khan, S. , Zada, S. , Ali, B. , and Kang, S. Pigment production by cold-adapted bacteria and fungi: colorful tale of cryosphere with wide range applications. Extremophiles 2020, 24, 447–473.

23) Loh, W. L. C. , Huang, K. C. , Ng, H. S. , and Lan, J. C. W. Exploring the fermentation characteristics of a newly isolated marine bacteria strain, Gordonia terrae TWRH01 for carotenoids production. Journal of Bioscience and Bioengineering 2020, 130, 187–194.

ISSN: 1673-064X
E-Publication: Online Open Access
Vol: 67 Issue 08 | 2024
DOI: 10.5281/zenodo.13358020

24) Kramar, A. , Ilic-Tomic, T. , Petkovic, M. , Radulović, N. , Kostic, M. , Jocic, D. , and Nikodinovic-Runic, J. Crude bacterial extracts of two new Streptomyces sp. isolates as bio-colorants for textile dyeing. World Journal of Microbiology and Biotechnology 2014, 30, 2231–2240.

25) El-Shall, F. N. , Fahim, A. M. , and Dacrory, S. Making a new bromo-containing cellulosic dye with antibacterial properties for use on various fabrics using computational research. Scientific Reports 2023, 13, 10066.

26) Nuanjohn, T. , Suphrom, N. , Nakaew, N. , Pathom-Aree, W. , Pensupa, N. , Siangsuepchart, A. , Dell, B. , and Jumpathong, J. Actinomycins from Soil-Inhabiting Streptomyces as Sources of Antibacterial Pigments for Silk Dyeing. Molecules 2023, 28, 5949.

27) Venil, C. K. , Dufossé, L. , Velmurugan, P. , Malathi, M. , and Lakshmanaperumalsamy, P. Extraction and Application of Pigment from Serratia marcescens SB08, an Insect Enteric Gut Bacterium, for Textile Dyeing. Textiles 2021, 1, 21–36.

28) Afshan, N. , Ullah, S. M. K. , and Kazmi, M. Evaluation of indigenously developed quick identification system (QTS-12) with API 10S. Proceedings of International Bhurban Conference on Applied Sciences and Technology 2018, 218–221.

29) Soundhari, C. and Haripriya, K. B. Extraction and characterization of antimicrobial pigment from marine bacteria and testing of antimicrobial activity against penicillin resistant. International Journal of Pharmaceutical Chemistry and Analysis 2022, 9, 50–55.

30) Masi, C. , Duraipandi, V. , Yuvaraj, D. , Vivek, P. , and Parthasarathy, N. Production and Extraction of Bacterial Pigments from Novel Strains And Their Applications. Biological and Chemical Sciences 2014, 5, 584–59.

31) Yang, L. , Cui, S. , Ren, Y. X. , Guo, L. K. , Liu, H. H. , Zhang, Z. H. , Tu, L. X. , Wang, J. , and Li, X. T. Nitrogen removal and aggregation characteristics of Pseudomonas aeruginosa YL and its application capacity for ammonium-rich wastewater treatment. Journal of Water Process Engineering 2021, 43, 102260.

32) DeBritto, S. , Gajbar, T. D. , Satapute, P. , Sundaram, L. , Lakshmikantha, R. Y. , Jogaiah, S. , and Ito, S. ichi Isolation and characterization of nutrient dependent pyocyanin from Pseudomonas aeruginosa and its dye and agrochemical properties. Scientific Reports 2020, 10, 1542.

33) Kumar, S. , Pandey, P. , and Maheshwari, D. K. Reduction in dose of chemical fertilizers and growth enhancement of sesame (Sesamum indicum L.) with application of rhizospheric competent Pseudomonas aeruginosa LES4. European Journal of Soil Biology 2009, 45, 334–340.

34) Chaudhari, V. M. Optimization of the extraction parameters for the production of biopigment from the new isolate of distillery effluent. Journal of Scientific and Innovative Research 2013, 2, 1044–1051.

35) Samyuktha, S. and Mahajan, S. Isolation and identification of pigment producing bacteria and characterization of extracted pigments. International Journal of Applied Research 2016, 2, 657–664.

36) Usman, H. M. , Abdulkadir, N. , Gani, M. , and Maiturare, H. M. Bacterial Pigments and its Significance. MOJ Bioequivalence & Bioavailability 2017, 4, 285–288.

37) Zheng, X. , Wang, J. , Chen, Z. , Zhang, H. , Wang, Z. , Zhu, Y. , and Liu, B. A Streptomyces sp. strain: Isolation, identification, and potential as a biocontrol agent against soilborne diseases of tomato plants. Biological Control 2019, 136, 104004.

38) Alqahtani, F. S. Pyocyanin Pigment Extracted from Pseudomonas Aeruginosa Isolate as Antimicrobial Agent and Textile Colorant. International Journal of Scientific Research 2016, 5, 467–470.

ISSN: 1673-064X
E-Publication: Online Open Access
Vol: 67 Issue 08 | 2024
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39) Krishna, J. G., Basheer, S. M., Beena, P. S., and Chandrasekaran, M. In Marine Bacteria As Source of Pigment for Application As Dye in Textile Industry, 2008.

40) Nikel, P. I. , Chavarría, M. , Danchin, A. , and Lorenzo, V. de From dirt to industrial applications: Pseudomonas putida as a Synthetic Biology chassis for hosting harsh biochemical reactions. Current Opinion in Chemical Biology 2016, 34, 20–29.

41) Martin-Pascual, M. , Batianis, C. , Bruinsma, L. , Asin-Garcia, E. , Garcia-Morales, L. , Weusthuis, R. A. , Kranenburg, R. van , and Martins dos Santos, V. A. P. A navigation guide of synthetic biology tools for Pseudomonas putida. Biotechnology Advances 2021, 49, 107732.

42) Loeschcke, A. and Thies, S. Pseudomonas putida—a versatile host for the production of natural products. Applied Microbiology and Biotechnology 2015, 99, 6197–6214.

43) Petruk, G. , Roxo, M. , Lise, F. De , Mensitieri, F. , Notomista, E. , Wink, M. , Izzo, V. , and Monti, D. M. The marine Gram-negative bacterium Novosphingobium sp. PP1Y as a potential source of novel metabolites with antioxidant activity. Biotechnology Letters 2019, 41, 273–281.

44) Alihosseini, F. , Ju, K. S. , Lango, J. , Hammock, B. D. , and Sun, G. Antibacterial colorants: Characterization of prodiginines and their applications on textile materials. Biotechnology Progress 2008, 24, 742–747.

45) Cheng, K. C. , Hsiao, H. C. , Hou, Y. C. , Hsieh, C. W. , Hsu, H. Y. , Chen, H. Y. , and Lin, S. P. Improvement in Violacein Production by Utilizing Formic Acid to Induce Quorum Sensing in Chromobacterium violaceum. Antioxidants 2022, 11, 849.